Brainstem Inflammation Associated with Septicemia Alters Ventilatory Pattern Variability in Rodents

TE Dick[1], FJ Jacono[1&2], KA Loparo[3], Y Molkov[4], Y Vodovotz[5], N Solanki[1], RY Santiago[1], D Nethery[1], and Y-H Hsieh[1]

[1]Division of Pulmonary, Critical Care & Sleep Med, Dept Med, Case Western Reserve Univ.; [2]Louis Stokes Cleveland VA Medical Center, [3]Dept Elect Eng & Computer Science, Case Western Reserve University; [4]Dept Math&Stat, Georgia State Univ; 5Dept of Surgery, Univ Pittsburgh

Introduction

- Sepsis is defined as a dysregulated host response to infection causing life-threatening organ dysfunction.
- Identifying sepsis as it develops is critical for successful treatment.
- We theorized that uncontrolled inflammation results from neural inflammation affecting brainstem control of cardiorespiratory function.

Hypothesis

- Specifically, brainstem inflammation leads to the loss of ventilatory pattern variability and uncouples cardioventilatory rhythms.

Methods

Experimental Design:

- Procedures were approved by the CWRU IACUC.
- Rats (Sprague-Dawley/Harlan, Male, n=16, 250-275g, male) were anesthetized with isoflurane and implanted with agar clots that were either sterile (n=3) or contained *Escherichia coli* (n=13) in the abdomen superficial to the peritoneum. Varying doses of *E. coli* were created (x10^6): 1 (n=3), 10 (n=3), 25 (n=3), 50 (n=3), 100 (n=1).

Inflammatory Cytokines Measurement:

- At 24h, the rats were euthanized by anesthetic overdose (Isoflurane). We harvested peripheral tissue and central nervous system structures:
  - Peripherally: Lungs, Broncho-Alveolar Lavage Fluid (BALF), Liver, Heart and Serum
  - Centrally: Pons, dorsal Medulla, ventral Medulla, Cervical Spinal Cord, Cerebellum and Cerebrum
- To measure inflammatory markers:
  - Tissues samples were ground in lysing buffer and centrifuged.
  - Supernatants were analyzed for IL-1b and TNFa using an enzyme-linked immunosorbent assay (ELISA). Cytokine levels were derived from known standards analyzed concomitantly.

Variability Analyses:

- Mean ± standard deviation (SD) and coefficient of variation (CV=SD/mean) of breathing frequency, (f_R, Brths/min). Epochs were determined by the number of cycles, 100 < n ≤ 200 cycles.
- Poincaré plots, cycle duration (T_{TOT}, s) n+τ, (τ =1 to 50 cycles) was plotted against n. We calculated indexes related to short- and long-term variability, (SD1 & SD2), as well as measures of Temporal Poincaré Variability (TPV_A & TPV_{TD}).
- Surrogate data sets (n=19) were generated using the iterated amplitude adjusted Fourier transform (iAAFT) moving the data to the frequency domain and back to the time domain and maintaining the frequency and amplitude distribution (autocorrelation function) of the original data set.
- Autocorrelation of the ventilatory waveform was generated for τ =1 to 1-cycle length. The r value at 1-cycle length was used as an index of the strength of the autocorrelation
- Mutual Information (MI) quantifies statistical dependence of a time-delayed coordinate *x(t+τ)* for τ =1 to 1-cycle length based on the coordinate *x(t)* and average value for MI within 17.5% of the minimum and maximum lags was calculated.
- Sample Entropy (SampEn) is a measure of the predictability of a signal. To calculate sample entropy, a template of m (m=2) points is selected and the entire epoch is searched for template matches within a certain tolerance r (r = 0.2*SD), let this value be A. The procedure is repeated with a template of m+1 (3) points, let the number of such matches be equal to B. SampEn is defined as |ln(A/B)| where self-counting is eliminated.
- Nonlinear complexity index is difference in SampEn between the original and the mean of the surrogate data sets.

Results

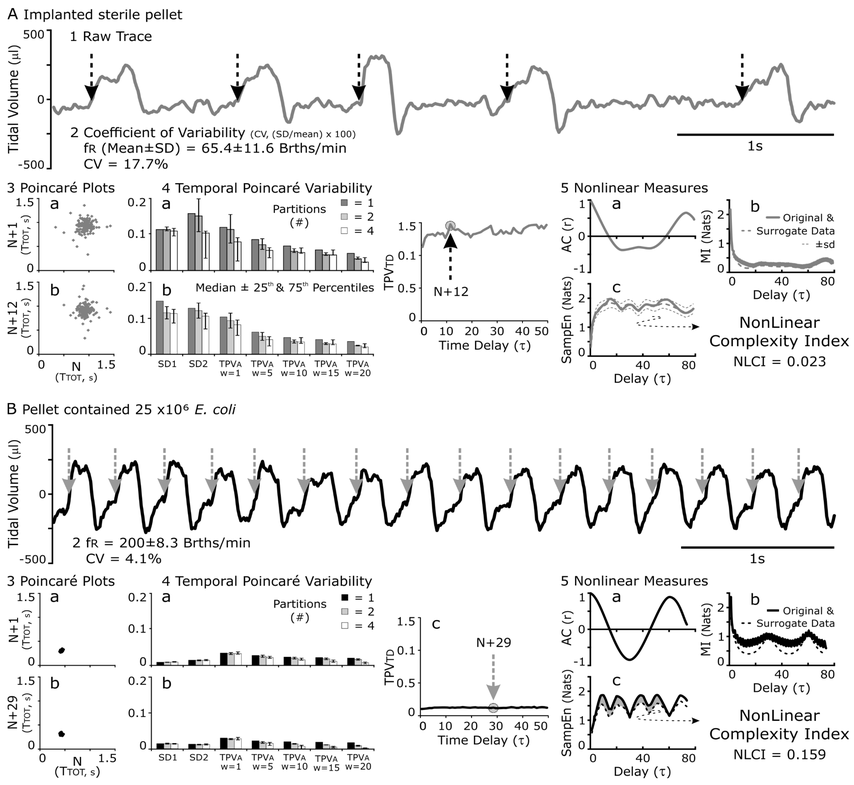

Figure 1: Ventilatory pattern analysis of a rats implanted with a sterile clot (**A**) or with an *E coli* infected clot (**B**).

- **1** Inspiration is up and arrows mark the onset of inspiration in raw traces.

In comparing the inoculated (**B**) to surgical control rat (**A**):

- **2** CV was less,
- **3** The distribution of points in Poincaré plots was smaller,
- **4** TPA_A (1A4a&b) and TPV_{TD} (1A4c) reflected less variability from the origin and from the line of identity,
- **5** AC increased; MI decreased; and SampEn, decreased; - indicated a more predictable ventilatory pattern for inoculated rats.
- **5** NLCI increased indicating a greater component of deterministic nonlinear type of variability

- In conclusion: A rat implanted with pellet infected *E. coli* increased its breathing frequency decreased its VPV compared to an uninoculated rat

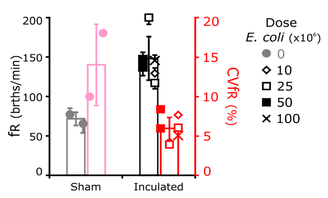

Figure 2. fR and CVfR for sham uninfected (gray) and inoculated (black) rats. In the infected rats, fR increased and CVfR decreased.

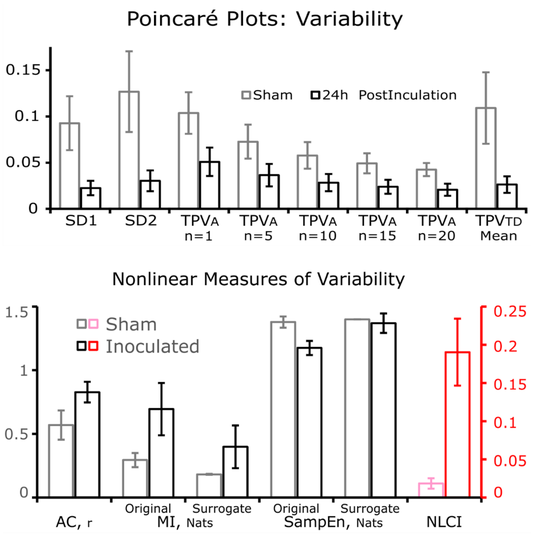

Figure 3. The magnitude of distribution of values decreased in Poincaré Plots. TPV_A decreased as 'n' increased, indicating that neighboring points were throughout the cloud.

Figure 4. Nonlinear assessments reflect **predictability** rather than variability of the waveform, AC and MI *increase* and SampEN *decrease* in the infected rat. NLCI reflects the *increased* deterministic variability in VPV.

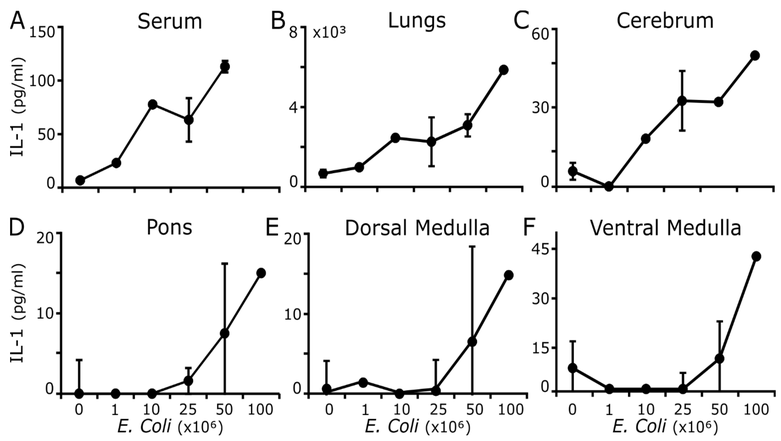

Figure 5. Group data for levels of IL-1b. The expression of Il=1b appeared to depend on the magnitude of *E. coli* in the clot. Il=1b increased with increasing Ecoli in peripherally in fluid and tissues as well as centrally in the cerebrum and in structures associated cardiorespiratory control.

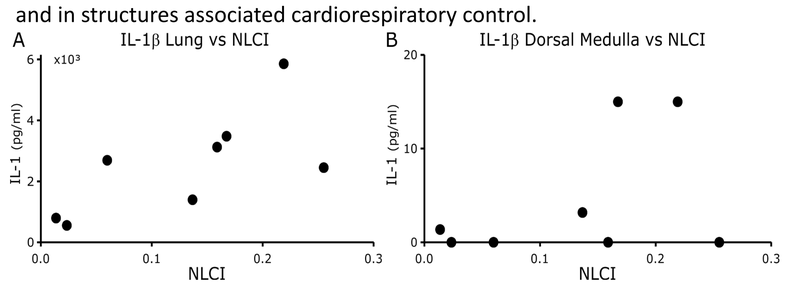

Figure 6. Preliminary correlations between NLCI and IL-1β.

- **A** The positive correlation between lung inflammation and VPV indicates that lung injury may be a central component in driving the decrease in VPV.
- **B** The tendency (*at best*) of a positive correlation between brainstem inflammation and NLCI is neither supports nor reflects the hypothesis.

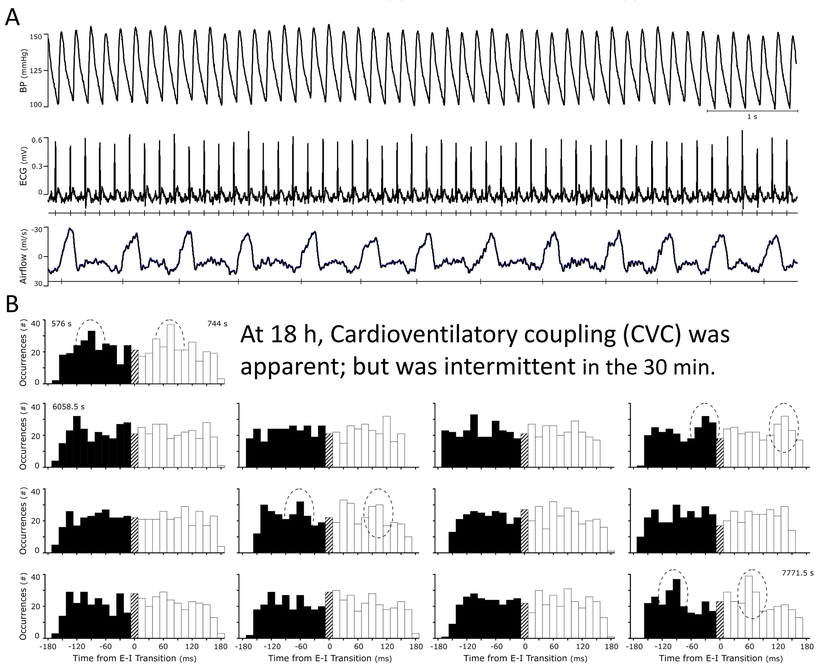

Figure 7. Preliminary data on the cardioventilatory coupling .

- **A** Raw data, Traces: Blood pressure, Electrocardiogram; Plethysmograph.
- **B** CardioVentilatory Coupling weakening – although the last epoch it returned.

Conclusions

- The battery of complementary analytical tools reflected VPV effectively.
- VPV decreased significantly in infected animals with systemic inflammation.
- But at the 24 time point, the VPV measures were not dose dependent.
- Inflammatory cytokines were measured using a standardized technique however, the zero values in the dorsal Medulla as unexpected in infected rats as our previous studies indicated it should be present.
- Refine the extraction of cytokines from the CNS tissue.
- More experiments are required.

Acknowledgements

Funded by: U01 EB021960 and a VA Research Award I01BX004197 to FJJ